REMARKS

Claims 9-23, 25 and 26 were pending in the application. Upon entry of these amendments, Claims 9-23, 25, and 26 will be pending and under active consideration. Claims 9 and 20 are independent.

Applicants respectfully request entry of the remarks made herein into the file history of the present invention. Reconsideration and withdrawal of the rejections set forth in the above-identified Office Action are respectfully requested.

I. The Rejections Under 35 U.S.C. § 103(a) Should Be Withdrawn

A. The Rejection Over Published Patent Application WO 95/31553 To Kossman *et al.* *In View of* Okada *et al.*

The Office Action, at pages 3-6, rejects Claims 9-15, 20-23, 25 and 26 as allegedly being obvious over Published Patent Application WO 95/31553 to Kossman *et al.* (hereinafter, "Kossman") in view of Okada *et al.* (J. Biol. Chem. 249(1):126-135 (1974)) (hereinafter, "Okada"), under 35 U.S.C. § 103(a). The Office Action alleges that Kossman discloses a process of preparing insoluble polysaccharides by contacting sucrose with an amylosucrase under aqueous conditions, that the enzyme should be obtained from the claimed microorganism, *Neisseria polysaccharea*, can be produced recombinantly, can be used in purified form, and can be used in immobilized form. While the Office Action acknowledges that Kossman differs from the cited claims in that Kossman does not employ the enzyme under buffer-free conditions, the Office Action alleges further that Kossman clearly discloses that the enzyme is useful at neutral conditions, for example at pH 6.5 (50 mM sodium citrate buffer).

Thus, the Office Action alleges that the artisan of ordinary skill, recognizing from Kossman that the enzyme is active at neutral conditions *which do not require the addition of a buffer*, clearly would have been motivated to have omitted the step of adding a buffer to the reaction medium disclosed by Kossman in order to make the process easier and cheaper by omitting the expense of a buffer. The Office Action alleges further still, that the artisan of ordinary skill clearly had a reasonable expectation that the process would work in the absence of a buffer, based on the fact that the Kossman discloses the enzyme as functioning at neutral conditions. The Office Action alleges further still that Kossman also differs from the claims as amended in that Kossman does not disclose that 70% of the sucrose is converted to α-1,4-glucans and fructose within 23.5 hours. However, the Office Action asserts that Okada discloses that the claimed enzyme produces an 80% conversion within 2 hours, a time much shorter than that recited in the claims, under the conditions assayed therein. Thus, the Office Action asserts that the artisan of ordinary skill clearly would have reasonably expected Kossman's process to have yielded a similar conversion, even in the absence of a buffer. In this regard, the Office Action notes that there is nothing in either Kossman or Okada suggesting that omission of buffer would lower enzyme efficiency. Applicants traverse respectfully.

Kossman relates to DNA sequences coding for proteins having the enzymatic activity of an amylosucrase which allows the synthesis of linear α-1,4 glucans from the substrate by bacteria, fungi and plants or in cell-free systems. Kossman furthermore discloses plasmids and bacteria containing these DNA sequences as well as processes for the production of plants and microorganisms capable of intracellullary or extracellulary expressing a polypeptide having amylosucrase activity. Kossman also discloses the production of pure fructose using proteins exhibiting the enzyme activity of amylosucrase.

As acknowledged by the Examiner, Kossman does not disclose the use of the enzyme under buffer-free conditions (Kossman at page 35 employs 50 mM sodium citrate buffer, pH 6.5). However, contrary to the Examiner's assertions, the use of a buffer is not necessarily to adjust the pH of a solution to neutral conditions, but primarily to keep an arbitrary pH value constant or at least nearly constant while an enzymatic reaction takes place. Furthermore, a buffer may also be needed to adjust the osmolarity of a reaction solution. One skilled in the art would not perform an enzymatic reaction without a buffer, because the activity of most enzymes is known to be strictly dependent on a constant pH value. In view of the fact that a buffer is generally necessary to maintain a specific pH, Applicants respectfully submit that it would not be obvious that the claimed reaction would work in the absence of a buffer. Applicants respectfully submit that, according to the present invention, there is a considerable advantage in performing the reaction in plain water because this results in significantly improved purity of the product. This obviates the need to subsequently remove residual buffer salts from the biotransformation products, α-1,4-glucans and fructose, the latter of which being of high purity may be used to produce high fructose syrups (HFS)).(Specification at page 4, lines 12-37). Therefore, the present invention is not taught or suggested by Kossman, as Kossman does not disclose the use of an enzyme under buffer-free conditions. serge

Applicants respectfully submit that, indeed, contrary to the Examiner's contention, it should be abundantly clear that the state of the art calls for use of a buffer in the enzymatic reaction for amylosucrase activity. Yet, while omitting the normally required buffer, the claimed invention still achieves a conversion rate of at least 70% of said sucrose to α-1,4-glucans and fructose within 23.5 hours. Applicants respectfully submit that this is a surprising and

unexpected result given the use of a buffer by those skilled in the art of amylosucrase enzymatic activity assays.

Moreover, Applicant respectfully asserts that there is nothing in the references of record, which would point one of ordinary skill in the art to perform the method of preparing water-insoluble α-1,4-glucans using amylosucrase in the absence of a buffer.

Thus, while the Office Action is correct in asserting that Kossman clearly discloses that the enzyme having amylosucrase activity is useful at neutral conditions, for example at page 35 wherein the enzyme is employed at pH 6.5, Applicants submit respectfully that the Office Action is in error when it draws from this teaching the inference that one skilled in the art would expect that maintenance of neutral conditions in a reaction solution does not require the addition of a buffer. Applicants submit respectfully that the skilled artisan would recognize that, while purified water indeed has a neutral pH, once reaction components are added to purified water, the pH of the aqueous solution changes. Applicants submit respectfully that the skilled artisan would recognize that a buffer is used, specifically and intentionally, to create and maintain given pH conditions in a reaction solution-- neutral conditions in this case-- that would otherwise not exist or remain at the given pH owing to the constituents and products of the given chemical reaction occurring in the solution. Applicants submit respectfully that one skilled in the art would be expected, further, to recognize that the presence of an ongoing chemical reaction in the aqueous solution would tend to drive the pH of the solution even further away from neutral conditions.

Applicants submit respectfully that one skilled in the art would recognize that, in enzymatic reactions, reaction efficiency is degraded substantially, or decreases to zero, when the reaction conditions vary from the optimum conditions recommended for the reaction. In order to

maintain the recommended optimum reaction conditions, for example to prevent harmful variations in pH, the skilled artisan routinely employs a suitable buffer.

In fact, Applicants respectfully submit that even in the reference of Okada, cited by the Examiner (Okada *et al.* (J. Biol. Chem. 249(1):126-135 (1974)) in support of the reasoning that Okada discloses that the claimed enzyme produces an 80% conversion within 2 hours under the conditions assayed therein (Fig. 2, page 128, top of right hand column), Okada performs the amylosucrase assay reaction in 0.05 M sodium maleate buffer, pH 6.4.

Indeed, in support of the basic notion that the skilled artisan routinely employs a suitable buffer in most enzymatic reactions, Applicants respectfully submit that references in the enzymatic field specifically directed to assaying the activity of amylosucrase have continued, and will no doubt continue, to employ a buffer in the amylosucrase assay.

For example, and not by way of limitation, Applicants respectfully direct the Examiner's attention to the reference of MacKenzie *et al.* (Can . J. of Microbiol. Vol. 23, 1977 pp. 1303-1307, cited by applicant as reference "AV2" in the information disclosure statement filed December 21, 2000), wherein MacKenzie *et al.* teach on page 1304, second column, first full paragraph, that "[t]he various fractions thus obtained were assayed for amylosucrase activity. Assay mixtures typically contained ... 25 ul of 50 mM sodium cacodylate buffer, pH 6.7." MacKenzie is also significant in its teaching at page 1305, first column, first full paragraph, that "[e]nzyme activity was optimal over quite a narrow pH range (pH 6.7-7.0) and was significantly inhibited by Tris-HCL buffers. Thus, MacKenzie *et* al. themselves indeed saw the importance of the use of a buffer in their amylosucrase assay to maintain the pH in the narrow range of pH 6.7-7.0 (albeit a sodium cacodylate buffer but <u>not</u> a Tris-HCL-based buffer).

Applicants also respectfully direct the Examiner's attention to the reference of Remaud-Simeon *et al.* (Carbohydrate Bioengineering 1995:313-320) cited by the Examiner in support of the rejection of claims 9-23, 25 and 26 to be discussed *infra*, wherein Remaud-Simeon teaches on page 315 under Section 2.4 "Reactions Conditions" that "[r]eactions were all conducted in 50 mM sodium maleate buffer, pH 6.4. at 30 C with various concentrations of sucrose."

Applicants also respectfully direct the Examiner's attention to the reference of Tao *et al.* (Carbohydrate Research 181 (1988) 163-174), wherein Tao *et al.* teach on page 166 under the Section entitled "Reactions Conditions" that "[a]ll reactions were all conducted at 35 C in in 50 – 100 mM sodium maleate, pH 7.0., containing 0.02% sodium azide."

Finally, Applicants also respectfully direct the Examiner's attention to the reference of Albenne C. *et al.* (J Biol Chem. 2004 Jan 2; 279(1): 726-34. Epub 2003 Oct 21, a copy of which is provided herewith as Exhibit A for the convenience of the Examiner), wherein Albenne C. *et al.* teach on page 728 first full paragraph under the Section entitled "Kinetics of Soluble Compound Formation from 100 mM Sucrose Using Wild-type AS" that "[r]eaction in the presence of 100 mM sucrose was carried out at 30 C in PBS buffer at pH 7.3, using pure wild-type AS at 375 mg/liter."

Indeed, a careful reading of Kossmann, Okada, MacKenzie, Remaud-Simeon, Tao, and Albenne does not reveal any instance of an assay of amylosucrase enzymatic activity conducted in the absence of a buffer. If, as suggested by the Examiner, one of ordinary skill in the art would have found it obvious, and indeed would have been motivated to conduct the amylosucrase reaction in the absence of a buffer as presently claimed, then Applicants respectfully ask why such a reaction has not been employed in the assay of amylosucrase activity in the scientific community even as late as the Albenne reference of 2004?

The Examiner has provided no reasoning or evidence that one of ordinary skill would have performed the amylosucrase reaction in the absence of buffer as in the claimed invention. Rather, Applicants respectfully submit that Kossman is combined with Okada to demonstrate only that the claimed enzyme produces an 80% conversion within 2 hours under the conditions assayed therein. This assertion, however, does not in any way address the fundamental deficiency of Kossman with respect to conducting the amylosucrase reaction in the absence of a buffer. Hence, Applicants respectfully conclude that the claimed invention itself has served as a "blueprint" for the Examiner's obviousness rejection.

The courts have made it clear that the disclosure of one reference can be properly combined with the disclosure of one or more additional references *only if* the requisite motivation for the combination can be found in the reference itself. As stated by the Federal Circuit:

> In proceedings before the Patent and Trademark Office, the Examiner bears the burden of establishing a prima facie case of obviousness based upon the prior art. . . . "[The Examiner] can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references." *In re Fine*, 837 F.2d 1071, 1074, 5 USPQ 2d 1596, 1598 (Federal National Mortgage Association. Cir. 1988).

In re Fritch, 23 USPQ 2d 1780, 1783 (Federal National Mortgage Association. Cir. 1992). It is also well-settled that a *prima facie* case of obviousness cannot be predicated on hindsight reconstruction. As stated by the Board:

> In the instant application, the examiner has done little more than cite references to show that one or more elements or subcombinations thereof, when each is viewed in a vacuum, is known. The claimed invention, however, is clearly directed to a combination of elements. That is to say, appellant does not claim that he has invented one or more new elements but has presented

> claims to a new combination of elements. To support the conclusion that the claimed combination is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed combination or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references. . . . Based upon the record before us, we are convinced that the artisan would not have found it obvious to selectively pick and choose elements or concepts from the various references so as to arrive at the claimed invention without using the claims as a guide. It is to be noted that simplicity and hindsight are not proper criteria for resolving the issue of obviousness.

Ex parte Clapp, 227 USPQ 972, 973 (B.P.A.I. 1985). Similarly, the Federal Circuit has stated:

> It is impermissible to use the claimed invention as an instruction manual or "template" to piece together the teachings of the prior art so that the claimed invention is rendered obvious. This court has previously stated that "[o]ne cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." (quoting *In re Fine*, 837 F.2d 1071, 1075, 5 USPQ 2d 1596, 1600 (Federal National Mortgage Association. Cir. 1988).

In re Fritch, 23 USPQ 2d 1780, 1784 (Federal National Mortgage Association. Cir. 1992). In the present case, the Examiner admits that the Kossman reference does not explicitly teach employing the enzyme under buffer-free conditions. Because one of ordinary skill in the art would not have been motivated to conduct an amylosucrase enzymatic reaction in the absence of a buffer simply for the common art-based practice of specifically including buffers to control pH and/or osmolarity variations, it would seem to Applicants that the Examiner's rejection is motivated instead by hindsight reasoning. Applicant recognizes that it may sometimes be difficult to guard against the insidious effects of hindsight reasoning, but it is incumbent upon the Examiner to resist the urge to use the Applicant's own disclosure to find a motivation to combine the teachings of the state of the art. Thus, the Examiner's rejection is apparently based on

hindsight reasoning and, thus, is improper in the first instance. The Examiner is respectfully reminded that at least each of the MacKenzie, Remaud-Simeon, Tao, and Albenne references specifically teach the utilization of a buffer for the amylosucrase reaction.

In the Office Action, at page 6, the examiner additionally asserts that "there is nothing in either Kossman or Okada suggesting that omission of buffer would lower enzyme efficiency." Applicants submit respectfully that this assertion is also improper because, for a finding of obviousness, a reference must teach or suggest positively that a modification from the taught method will work. As the examiner is no doubt well aware, a teaching or suggestion that a modification will not work is known as teaching away. Applicants submit respectfully that failure in a reference to teach away from a modification does not render that modification obvious over the reference.

"To support the conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references." *Ex parte Clapp*, 227 USPQ 972, 973 (Bd. Pat. App. & Inter. 1985). Applicants submit respectfully that Kossman, either alone or in combination with Okada, does not expressly or impliedly suggest the claimed invention, and Applicants further submit respectfully that the Examiner has not presented a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references. Inasmuch as the present 35 U.S.C. § 103(a) rejection of Claims 9-15, 20-23, 25 and 26 is based solely on Examiner's unsupported assertion that one skilled in the art would have been motivated to exclude from his reaction the buffer disclosed in Kossman or Okada, despite the fact that

Kossman or Odada makes no teaching or suggestion that the reaction would be expected to succeed in the absence of buffer, Applicants respectfully request that Examiner provide a citation or reference in support of Examiner's assertion so that Applicants will have free and fair opportunity to rebut Examiner's argument, as required under MPEP 2144.03. The Examiner is also respectfully reminded that at least each of the MacKenzie, Remaud-Simeon, Tao, and Albenne references specifically teach the utilization of a buffer for the amylosucrase reaction.

"In determining the propriety of the Patent Office case for obviousness in the first instance, it is necessary to ascertain whether or not the reference teachings would appear to be sufficient for one of ordinary skill in the relevant art having the reference before him to make the proposed substitution, combination, or other modification." *In re Linter*, 458 F.2d 1013, 1016, 173 USPQ 560, 562 (CCPA 1972). Applicants submit respectfully that, in light of the well-known propriety of using buffers to maintain appropriate reaction conditions for enzymatic reactions, and the equally well-known costs for failure to maintain reaction conditions in enzymatic reactions (*i.e.*, loss of reaction efficiency and/or risk of complete reaction failure), one skilled in the art would not be motivated to attempt the reactions disclosed in either Kossman or Okada in the absence of the suggested buffer.

For the reasons cited above, the rejection of Claims 9-15, 20-23, 25 and 26 as allegedly being obvious Kossman in view of Okada under 35 U.S.C. § 103(a) cannot be sustained and should be withdrawn.

B. The Rejection Over Kossman In View Of Remaud-Simeon

At page 5-6, the Office Action rejects Claims 9-23, 25 and 26 under 35 U.S.C. § 103(a) as being unpatentable over Kossman in view of Okada *et al.* (J. Biol. Chem.

249(1) :126-135 (1974)), as applied to claims 9-15, 20-23, 25 and 26 above, and further in view of Remaud-Simeon (Carbohydrate Bioengineering 1995:313-320). While acknowledging that Kossman differs from Applicants' claimed invention in that Kossman does not disclose the addition of a polysaccharide acceptor which may be dextrin, glycogen or amylopectin, the Office Action alleges that Remaud-Simeon cures the deficiencies of Kossman by disclosing that glycogen, starch (which contains amylopectin) and maltooligosaccharides act to activate amylosucrase when they are added to the reaction medium. The Office Action alleges that, thus, the artisan of ordinary skill would have been motivated to have added glycogen and amylopectin to the reaction medium to have afforded the activating effect disclosed by Remaud-Simeon. Moreover, the Office Action alleges that, in view of the fact that dextrins are very similar chemically to the compounds disclosed by Remaud-Simeon as having an activating effect on amylosucrase, the artisan of ordinary skill would have had a reasonable expectation that dextrins would have had the same activating effect on amylosucrase as glycogen, starch (which contains amylopectin) and maltooligosaccharides, and so the Office Action alleges that the artisan of ordinary skill would, therefore, have been motivated to have added dextrin to the reaction medium used for the production of glucans by amylosucrase. Applicants traverse respectfully.

Kossman and Okada have been discussed at length above. Remaud-Simeon relates to the cloning of chromosomal *Sau* 3A DNA fragments from *Neisseria polysaccharea* into phage λ EMBL3 to characterize amylosucrase activity (E.C. 2.4.1.4.) and to evaluate its potential use as a glycosylation tool. A recombinant phage expressing the amylosucrase activity was isolated. Production of the enzyme was carried out by infection of liquid culture of *E. coli*. The enzyme was purified from culture lysate to a specific activity, and when incubated with sucrose and traces of glycogen, the recombinant amylosucrase produced an insoluble glucopolysaccharide

mainly composed of α-(1→4) glucosidic linkages and a very low degree of α-(1→6) branched linkages (less than 5%). The recombinant enzyme is activated by glycogen, starch and maltooligosaccharides. It also catalyzes the transfer of glucosyl residue from sucrose onto a maltopentaose acceptor to produce maltohexose and heptose.

Applicants respectfully submit that, as previously noted above, Remaud-Simeon teaches that reactions were all conducted in 50 mM sodium maleate buffer, pH 6.4. Thus, Remaud-Simeon does not cure the fundamental deficiency of Kossman, either taken alone or in combination with Okada. Inasmuch as the secondary reference Remaud-Simeon is not alleged to cure this fundamental deficiency in either Kossman or Okada, Applicants submit respectfully that a *prima facie* case for obviousness under 35 U.S.C. § 103(a) has not been established. Accordingly, Applicants request respectfully that the rejection of Claims 9-23, 25 and 26 under 35 U.S.C. § 103(a) be withdrawn.

II. Rejections Under 35 U.S.C. § 112, Second Paragraph

At pages 2-3 of the Office Action (Paper No. 20), Claims 14, 25, and 26 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to point out particularly and claim distinctly the subject matter regarded as the invention. The Office Action alleges that the recitations "a purity of at least 80%," "a purity of at least 90%," and "a purity of at least 95%," in claims 14, 25 and 26, are indefinite because it is not clear whether the recitation requires a weight percentage, a molar percentage, or a percentage of a particular specific activity. The Examiner has indicated that while the claims now recite the purity in terms of specific activity, and that this activity can be compared to the specific activity of pure amylosucrase, it is allegedly not clear what the specific activity of pure amylosucrase is,

and that the Applicants have not established what an art-recognized specific activity for pure amylosucrase. Applicants traverse respectfully.

Respectfully, Applicants once again direct Examiner's attention to page 7, lines 8-13, and Examples 1-3, on page 13-17 of the specification as filed, and in particular to Example 1, which teaches a method of purifying an amylosucrase enzyme of the present invention, and Example 2, which teaches the determination of the enzyme's specific activity. Page 7, lines 8-13, define the term "purified amylosucrase" as an amylosucrase that is substantially free from the cell constituents of the cells in which the protein is synthesized. A number of purification techniques are listed on page 10, lines 10-12, such as precipitation, affinity chromatography, ion exchange chromatography, gel filtration, reverse-phase HPLC, etc. Applicants submit respectfully that one skilled in the art will recognize immediately that these techniques are effective to separate a protein of interest from cellular debris, DNA, lipids, and other unwanted proteins. Likewise, Applicants submit respectfully that the protocol of Example 1 will be familiar to one skilled in the art as a method of purifying a protein of interest from a raw cell extract.

Moreover, Applicants again respectfully direct the Examiner's attention to Example 2, particularly lines 5-8 on page 15 of the specification as filed, which teach that protein purity is assessed in terms of the desired enzymatic activity; in this case, amylosucrase activity. As recited in lines 6-7 on page 15, a preferred measure of purity is U/μg (units of activity per microgram of purified protein). Applicants submit respectfully that one skilled in the art will understand that impurities in the protein product will reduce the ratio of the desired enzymatic activity relative to the total amount of protein product isolated. Thus, Applicants submit respectfully that one skilled in the art will be able to determine readily the purity of amylosucrase, expressed as a percentage of the specific activity of pure amylosucrase, by

comparing the known value of the specific activity of pure amylosucrase with the activity per microgram of protein product used in the methods of the present invention as claimed.

Notwithstanding the above, and without acquiescing in the propriety of the rejection of Claims 14, 25, and 26 under 35 U.S.C. § 112, second paragraph, Applicants respectfully submit that one skilled in the art to which the invention pertains would know that the specific activity of amylosucrase is indeed known. For example, and not by way of limitation, Applicants respectfully direct the Examiner's attention to the publication of M. Remeaud-Simon *et al.* (Remeaud-Simon, in Petersen, Svenson and Petersen (Eds.) Carbohydrate bioengineering; Elsevier Science B.V., Amsterdam The Netherlands (1995), pp. 313-320. In particular, in Section 2.4. under Reaction Condistions, it is taught that one unit of amylosucrase activity is the amount of enzyme that catalyzes the release of one μmole of fructose per min at 30°C in 50 mM sodium maleate buffer, pH 6.4.

On this basis, Applicants suggest respectfully that the rejection has been traversed, and Applicants request respectfully that the 35 U.S.C. § 112, second paragraph rejection of Claims 14, 25, and 26 be withdrawn.

CONCLUSION

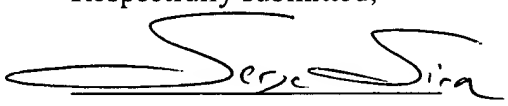
Applicants submit that the application is in condition for allowance. Favorable reconsideration, withdrawal of the rejections set forth in the above-noted Office Action, and an early Notice of Allowance are requested.

Applicants' undersigned attorney may be reached in our Washington, D.C. office by telephone at (202) 625-3500. All correspondence should be directed to our address given below.

AUTHORIZATION

Applicants believe there is no additional fee due in connection with this filing. However, to the extent required, the Commissioner is hereby authorized to charge any fees due in connection with this filing to Deposit Account 50-1710 or credit any overpayment to same.

Respectfully submitted,



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